As an application of this method we study a gene circuit with positive autoregulation that exhibits bistability. We show how the region of bistability becomes diminshed by increasing the effect of noise via a reduced copy number of the regulatory protein. Cell volume determines the region of bistability for different noise strength. The method is general and can also be applied to other cases where synthesis of proteins are regulated and an appropriate analytical description is difficult to achieve.

2833-Plat
Spatio-Temporal Measurements and Modeling of Genetic Expression
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Single-molecule, single-cell studies of genetic expression have provided key insights into how cells respond to external stimuli [Munsky, B., et al., Science (2012)]. By directly measuring copy numbers of individual bio-molecules in cells, it is now possible to obtain statistical measures of the spatio-temporal distributions of key signaling and regulatory networks. Such comprehensive datasets can be used to infer system-level models that yield quantitative insight into cellular regulation, predict cellular responses in new experimental conditions, and suggest more revealing experiments to uncover regulatory dynamics. The integration of single-molecule spectroscopy, biochemistry, and numerical modeling is a powerful multi-disciplinary approach to investigating cellular response at the genetic level.
A key issue we seek to address is what types of fluctuations are most informative about the underlying gene regulatory process. In other words, how much experimental resources should be spent to measure (i) temporal, (ii) spatial, or (iii) cell-to-cell fluctuations? As an example, we studied Interluekin 1-alpha (IL1α) mRNA expression within human THP-1 cells during stimulus response to lipopolysaccharide (LPS). By spatially resolving individual mRNA using multiplexed single molecule FISH [Femino A.M., et al., Science (1998), Raj A., et al., Nat Meth (2008)] in large populations of single cells at multiple times points, we quantified all three fluctuation types.
We expanded the common bursting gene expression model [Peccoud, J., Theoretical Population Biology (1995)] and derived a set of linear ODEs to describe the mean, variance, and co-variance of nuclear and cytoplasmic IL1α mRNA. We fit this model to multiple single-cell datasets. Comparing models inferred from each data set, we are able to draw conclusions on which fluctuation types are most revealing about the underlying system's mechanisms and parameters, providing feedback for new experiments. The approach developed here is applicable to any eukaryotic gene expression pathway.

2834-Plat
Gene Location and DNA Density Determine Transcription Factor Distributions in E. Coli
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The diffusion coefficient of the canonical transcription factor Lac Repressor, LacI, within living Escherichia coli has been measured directly by in vivo tracking to be D = 0.4 μm^2/s. At this rate, simple models of diffusion lead to the expectation that LacI and other proteins will rapidly homogenize throughout the cell.
We have tested this expectation of spatial homogeneity by high-throughput single molecule visualization of LacI molecules non-specifically bound to DNA in fixed cells to generate an ensemble average of the steady-state distribution of protein in the cell. Contrary to expectation, we find that the distribution of LacI depends on the spatial location of its encoding gene. We demonstrate that the spatial distribution of LacI is also determined by the local state of DNA compaction, and that E. coli can dynamically redistribute proteins by modifying the state of its nucleoid. We then show that LacI inhomogeneity increases the strength with which targets located proximally to the LacI gene are regulated. Finally, we propose a model for intranucleoid diffusion which can reconcile these results with previous measurements of LacI diffusion.

2835-Plat
Transcriptional Control by Regulated Oligomerization
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Numerous transcription factors self-assemble into different order oligomeric species in a way that is actively regulated by the cell. The functional role of this widespread process is not yet completely understood. Here we capture the effects of regulated oligomerization in gene expression with a novel quantitative framework. We show that this mechanism provides precision and flexibility, two seemingly antagonistic properties, to the sensing of diverse cellular signals by systems that share common elements present in transcription factors like p53, NF-κB, STATs, Oct, and RXR. Applied to the nuclear hormone receptor RXR, this framework accurately reproduces a broad range of classical, previously unexplained, sets of gene expression data and corroborates the existence of a precise functional regime with flexible properties that can be controlled both at a genome-wide scale and at the individual promoter level.

2836-Plat
Novel Mechanism for Noise Reduction between Nuclear Transcriptional Activity and Cytoplasmic MRNA Distributions Living Fly Embryos
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Noise in gene expression has been shown to play an important role in various organisms. In particular, transcriptional bursts are thought to be a common mode for mRNA output. Yet, the final patterns of gene expression, especially in the case of multicellular organisms such as Drosophila melanogaster, are highly precise and reproducible. In order to address this apparent contradiction, we present a strategy to measure transcriptional dynamics in living fly embryos by monitoring the production of mRNA at their sites of transcription on DNA loci in real time simultaneously in hundreds of individual nuclei. In particular, we measure the transcriptional activity of the hunchback promoter as a function of the position along the embryo throughout the first three hours of development. We observe "waves" of transcription associated with the progressing mitotic cycles, where transcription rises steadily in the beginning of the nuclear cycle, peaks mid-way through it and disappears during mitosis. Although the overall shape of these transcription waves is similar, we see a high degree of variability among nuclei. However, we see no evidence of transcriptional bursting. Single molecule mRNA FISH reveals that this variability in transcription rate does not translate into noise in the cytoplasmic mRNA distribution suggesting the need for a mechanism of noise rectification. In fact, we observe an overall 4-fold noise reduction between nuclear activity and cytoplasmic mRNA levels. using a simple stochastic model we demonstrate that our noise measurements can only be explained through a combination of both temporal and spatial averaging.

2837-Plat
Shaping Gene Expression in Artificial Cellular Systems by Cell-Inspired Molecular Crowding
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Synthetic biology has made tremendous recent strides in constructing artificial cellular systems using minimal cell components *ex vivo*, creating an experimental platform for characterizing the behavior of isolated cellular modules and a form of biotechnology for the controlled operation of artificial cells. The robustness and efficiency of these systems are nonetheless challenging to control, in part because artificial cells establish an environment that is still very different from that of actual living cells. Here, we present a novel approach towards bridging the gap between artificial and true cell environments by developing artificial cells incorporating controlled macromolecular crowding, mimicking a key feature of natural cells known to dramatically influence biochemical kinetics. We demonstrate the value of our approach by showing that molecular crowding enhances gene expression and confers robustness against perturbations of gene environments. We further elucidate the underlying mechanisms of these phenomena at the single molecule level by demonstrating how large crowding molecules decrease diffusion of T7 RNA polymerase, but increase its binding to a T7 RNAP promoter. Based on single-molecule results, we further show that the impact of molecular crowding on gene circuits is enhanced by weak genetic components and maximized by a negative feedback loop. By bridging a key gap between artificial cell technology and the environment of living cells, we demonstrate the importance of intracellular crowding to efficient and robust function of biological circuits and suggest new engineering principles for controlled modulation of synthetic genetic systems.

Platform: Protein Assemblies, Aggregates, & Chaperones

2838-Plat
Biophysical Analysis of a Novel Drug Delivery Vector: ELP[V5G3A2-150]
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